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# A Metabolic Model for the 0092 Morphotype Associated with Filamentous Bulking Problems in Wastewater Treatment Plants

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## BACKGROUND

Overgrowth of filamentous bacteria in activated sludge wastewater treatment plants (WWTPs) leads to impaired sludge settleability, a condition known as bulking, which is a common operational problem worldwide. The B45 genus-level-taxon, exhibiting the Eikelboom 0092 filament morphotype, is among the most abundant members of the phylum Chloroflexi in activated sludge (Fig. 1), yet nothing is known about their metabolic characteristics. In this study, we constructed a genome based metabolic model, that describes in detail the physiology of the organism in WWTPs, which was partly validated *in situ* with single cell methods.

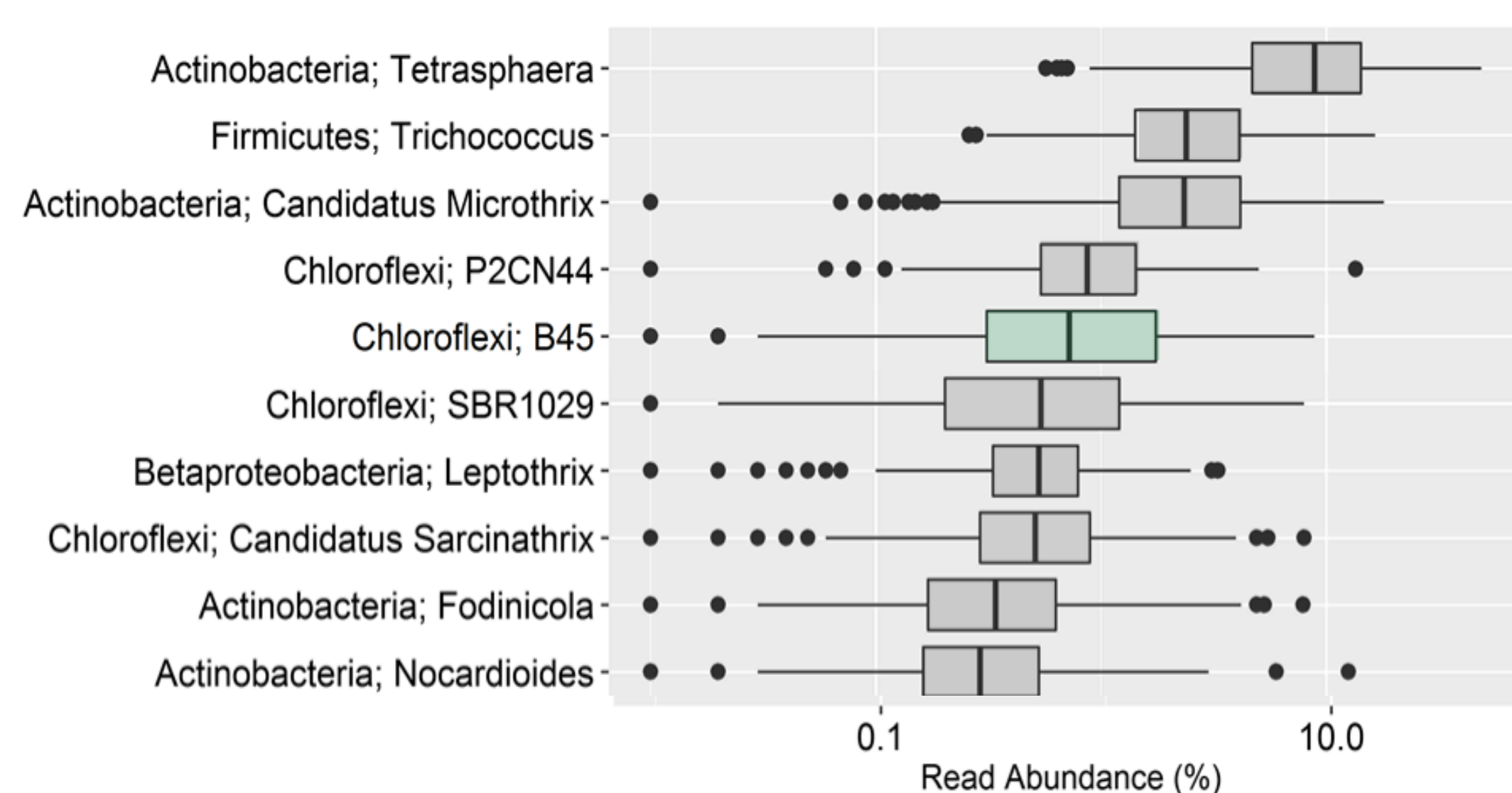
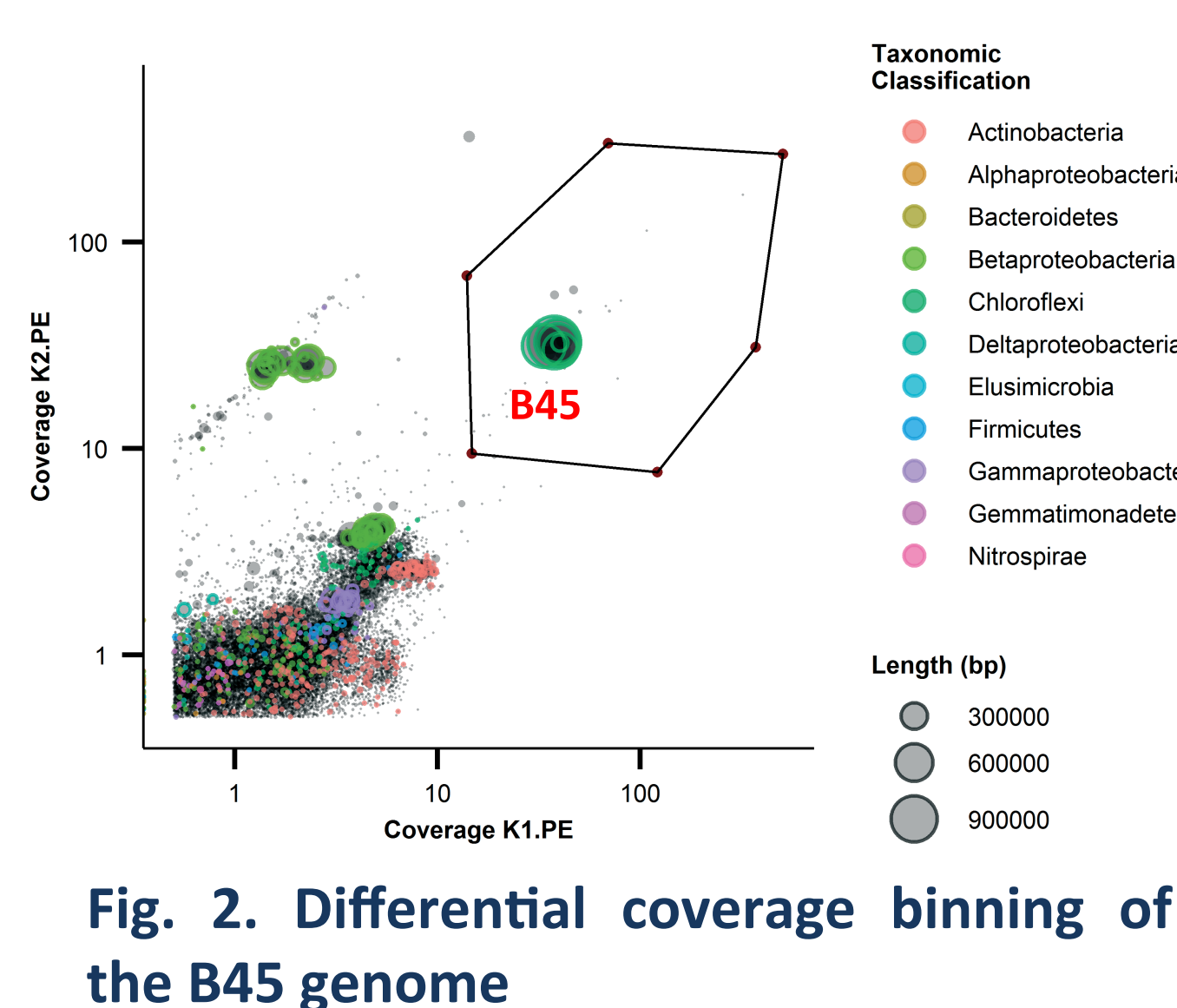


Fig. 1. Box plot showing the 10 most abundant genera known to have filamentous members. Abundances are based on the extensive MiDAS 16S rRNA gene amplicon survey of 24 full-scale nutrient removal plants in Denmark over 8 years (McIlroy *et al.*, (2015) Database. bav062.)

## METAGENOMIC ANALYSES

A metagenome was prepared from a WWTP suffering from severe 0092 morphotype associated bulking. A closed genome was assembled from the metagenome using differential coverage binning (Fig. 2) (Albertsen *et al.*, (2013) *Nature Biotech.* 31. 533-538)



The assembled genome was annotated with the Microscope platform (Vallenet *et al.*, (2009) Database. bap021).

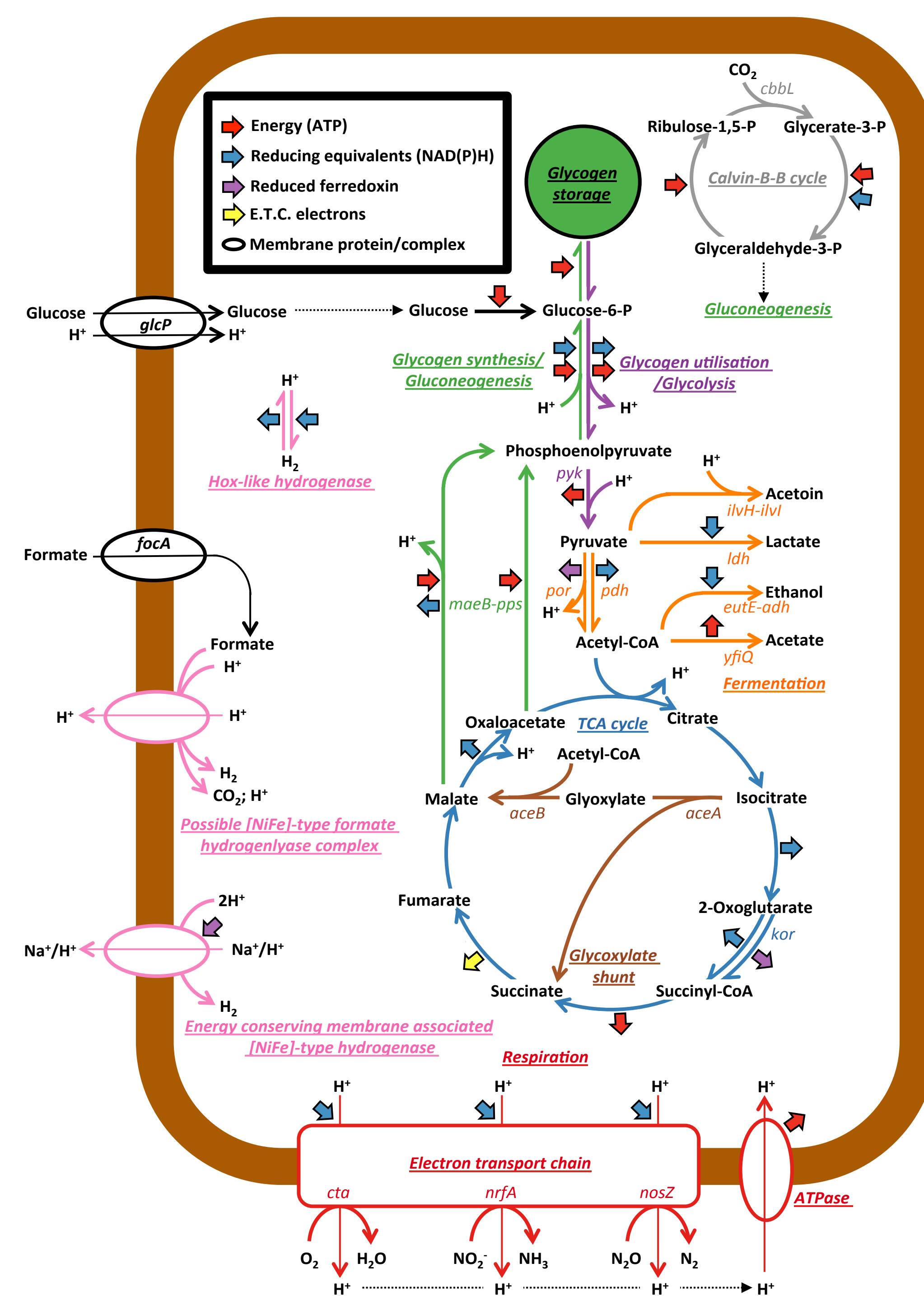
## STUDY PERSPECTIVES

In this study metagenomics and *in situ* methods were applied to construct a metabolic model for members of the B45 phylotype – revealing their role as abundant fermenters in activated sludge treatment systems. For more detail see McIlroy *et al.*, (2016) *ISME J.* 10. 2223-2234.

The genome obtained in this study provides the foundation for gene expression studies for more detailed insights into the *in situ* physiology of the B45 in WWTPs.

The multifaceted approach of this study provides a model for the systematic characterisation of the abundant organisms in full-scale systems.

## METABOLIC MODEL CONSTRUCTION



Based on the annotated genome and *in situ* analyses the metabolic model (Fig. 3) predicts:

- The B45 ferment sugars, when carbon is available under anaerobic conditions, with glycogen storage possible.
- Acetate, lactate, acetoin, ethanol and H<sub>2</sub> are potential by-products of sugar metabolism.
- O<sub>2</sub>, NO<sub>2</sub><sup>-</sup> and N<sub>2</sub>O are potential electron acceptors, indicating activity over a range of conditions.

## IN SITU MODEL VALIDATION

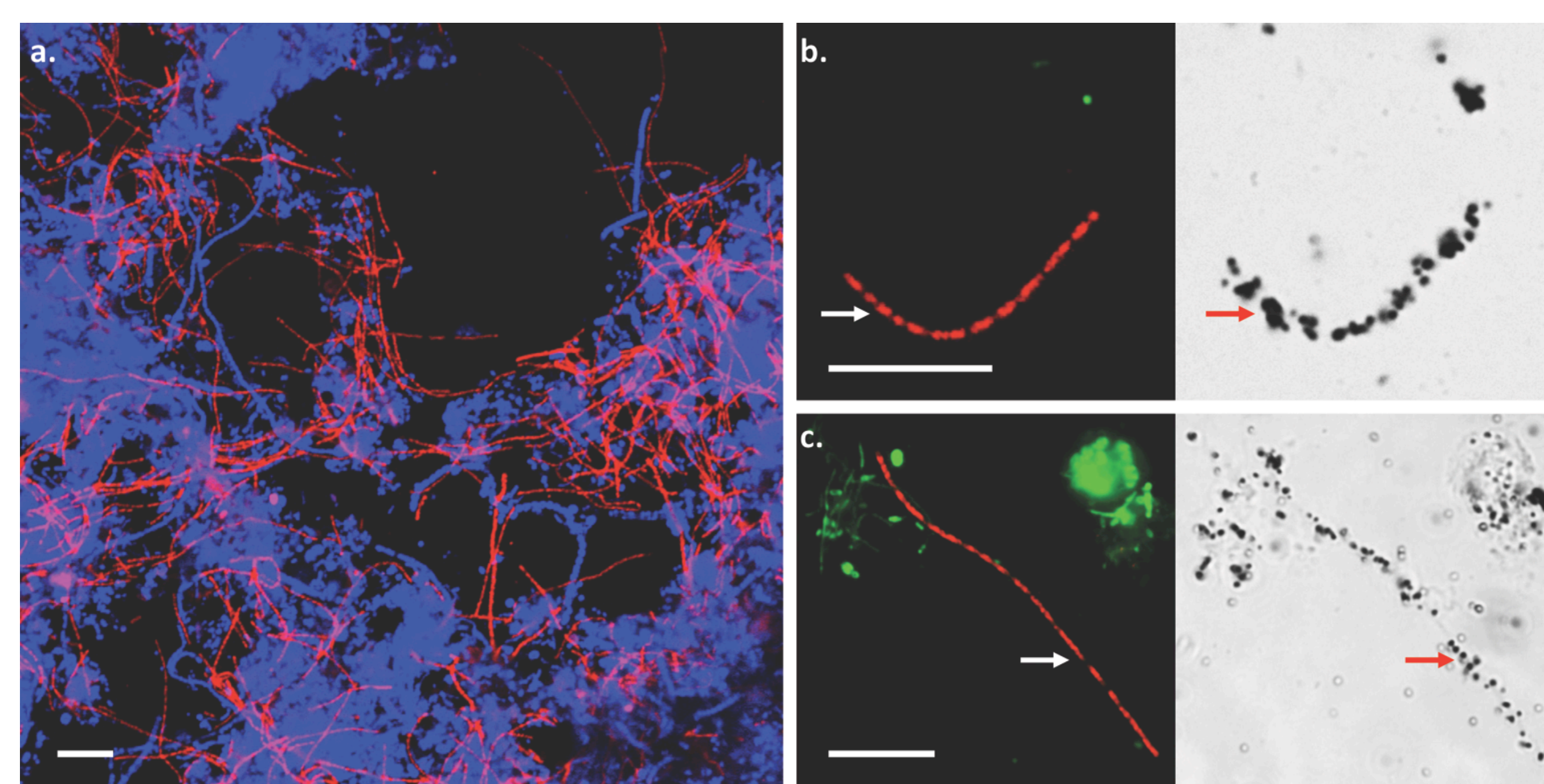


Fig. 4. FISH microscope images of the B45 phylotype. (a.) FISH image of the B45 (red filaments: CFX197 probe) in the WWTP used to prepare the metagenome. Other bacteria appear blue (EUBmix probe set). (b.) & (c.) FISH images of the B45 phylotype (red) and corresponding brightfield images of MAR signal (black silver granules) showing positive glucose uptake under (b.) aerobic and (c.) anaerobic conditions.

Fluorescence *in situ* hybridisation (FISH) was combined with microautoradiography (MAR) and staining to confirm key aspects of the model *in situ*. A fermentative metabolism was supported by anaerobic glucose uptake (Fig. 4c.)